LOW-LEVEL EFFECTS OF VX VAPOR EXPOSURE ON PUPIL DIAMETER AND CHOLINESTERASE LEVELS IN RATS

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ABSTRACT

The median effective concentrations (EC_{50}'s) for miosis in male and female rats exposed to VX vapor for 10, 60 and 240 min were estimated using whole body vapor exposures conducted in a 750 liter dynamic airflow inhalation chamber. Miosis was defined as at least a 50% reduction in pupil diameter relative to baseline measurements. Results show that the median effective dosages (ECt_{50}) for miosis are approximately an order of magnitude lower than the calculated ECt_{50} values for both GB and GF at each of the 3 exposure durations. There were significant gender differences in the ECt_{50} values for male and female rats with female rats being more sensitive to the effects of VX than males. There was significant whole blood AChE depression at the highest concentrations at each of the exposure times. The results of this study have identified several effects of VX vapor that could impact operational readiness and serve as a basis for predictions useful for military Operational Risk Management (ORM) decisions.

1. INTRODUCTION

O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX) is an extremely toxic organophosphorous (OP) compound which has been the subject of much research for over half a century. VX is more toxic than related anticholinesterase compounds such as sarin (GB), cyclosarin (GF), tabun (GA) and soman (GD). The bulk of what is known of the effects of VX on biological systems is derived from studies administering VX subcutaneously, percutaneously or intravenously. Few studies exist in which reliable toxicity estimates in animals have been established for VX administered as a vapor. To date, there are large information gaps regarding the toxicity effects from controlled exposures to VX vapor. Contributing to this lack of information is the difficulty in producing stable vapor concentrations in a controlled environment due to the very low vapor pressure of VX (0.00063 mm Hg @ 25°C compared to 2.9 mm Hg @ 25°C for sarin (GB)). In recent years, efforts to reduce the error currently embedded in various estimates of vapor toxicity and to refine various risk assessment tools has led to an interest in the low-dose effects of various OP compounds to include VX. Of specific interest are the threshhold concentrations of nerve agents below which there are no observable effects and above which more severe measurable effects are produced. Miosis is the "first noticeable effect" (FNE) for low-level concentrations of VX vapor and defining the ECt_{50}'s for miosis is important because of the military implications related to performance degradation and operational readiness.

2. METHODS

2.1 Exposure Protocol

Sexually mature male and female Sprague-Dawley rats weighing between 180 and 300 g were used in this study. Whole body vapor exposures were conducted in a 750-L dynamic airflow inhalation chamber. The rats were exposed for either 10, 60 or 240 min. Five concentrations of VX as well as sham controls were tested at each exposure duration.

2.2 Assessing Pupil Size

This study utilized a non-invasive method of assessing pupil size whereby projected infrared (IR) light (880 nm) reflected off the animal's retina back through the pupil producing an image of a bright pupil surrounded by a dark iris. The right eye of all rats in the study was digitally photographed on 3 different days prior to exposure in order to establish an average baseline pupil size. Pictures were also taken within 60 minutes post exposure, 2 hr, 24 hr, 48 hr and 7 days post exposure. All photographs were taken under low-light conditions (< 10 foot-candles).

2.3 Cholinesterase Inhibition Assay

Blood draws from the rats tail were done once before exposure, approximately 60 min post exposure and 7 days post exposure. Approximately 1 mL of blood was required for each draw.

The method used for measuring whole blood acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities was a modification of the Ellman

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Reference Method (Ellman *et al*., 1961). For determination of AChE activity, 25 µl of a solution containing 10mM acetylthiocholine and 200 µM 10-(α-diethylaminopropionyl)-phenothiazine, a specific inhibitor of butyrylcholinesterase (EQM Research, Cincinnati, OH), was added to the appropriate wells of the 96-well plate. For determination of BChE activity, 25 µl of a solution containing 20 mM butyrylthiocholine (EQM Research, Cincinnati, OH) was added to the appropriate wells of the 96-well plate. The plate was then read at 450 nm and 37°C using a SpectraMax Plus^{384} microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA) for 10 minutes, and analyzed using SoftMax Pro LS version 4.3 software (Molecular Devices Corp., Sunnyvale, CA). AChE activity values in whole blood were expressed as Units of activity per gram of hemoglobin (U/g HGB). BChE activity values in whole blood were expressed as Units of activity per gram of total plasma protein (U/g TPP).

3. RESULTS

The VX ECt_{50} values for miosis (Table 1) are approximately an order of magnitude lower than the calculated ECt_{50} values for both GB (Mioduszewski *et al*., 2002) and GF (Whalley *et al*., 2004) at each of the 3 exposure durations. For male rats, VX was 8-13 times more potent than GB and 11-18 times more potent than GF. For female rats, VX was approximately 9-11 times more potent than GB and 11-15 times more potent than GF. There were significant gender differences between the ECt_{50} values at each of the exposure duration times. Female rats appear more sensitive to the effects of VX than males. There was significant AChE depression at the highest concentrations of each exposure time. Butyrylcholinesterase levels in whole blood showed no significant depression.

Table 1. Miosis Level ECt50 Values for VX Vapor Exposures

VX			95% Fiducial Interval			95% Fiducial Interval	
		EC_{50}	Lower	Upper	ECt_{50}	Lower	Upper
Sex	Time (min)	mg/m^3	Limit	Limit	mg-min/m^3	Limit	Limit
m	10	0.01	0.0085	0.0124	0.102	0.085	0.124
m	60	0.004	0.0030	0.0050	0.229	0.180	0.300
m	240	0.002	0.0015	0.0023	0.443	0.363	0.547
f	10	0.007	0.0060	0.0089	0.073	0.060	0.089
f	60	0.002	0.0014	0.0023	0.106	0.087	0.136
f	240	0.001	0.0009	0.0014	0.268	0.219	0.326

4. DISCUSSION

These newly established ECT_{50}'s for VX may reduce the need for relative potency analysis using other nerve agents such as GB to establish toxicity levels for VX inhalation exposures. The need to establish separate ECT_{50}'s for males and females at each exposure duration is the result of the female rats being significantly more sensitive to VX. There are numerous studies which show that the actions of a variety of other drugs are more pronounced and/or persist longer in female rats than in male rats (Kato, 1974). This differential sensitivity appears to be mediated in part by androgens such as testosterone which increase the activities of drug-metabolizing enzymes in male rat-liver microsomes.

A highly selective binding affinity of VX for AChE (Maxwell, 1992) may explain the significant depression of AChE but not BChE after exposure to low doses of VX.

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